

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Lisa MCKERRACHER et al.

Application No.: NEW

Confirmation No.: N/A

Filed: March 28, 2006

Art Unit: N/A

For: CLOSTRIDIUM BOTULINUM C3
EXOTRANSFERASE COMPOSITIONS AND
METHODS FOR TREATING TUMOUR
SPREADING

Examiner: Not Yet Assigned

LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The PTO is requested to use the amended sheets/claims attached hereto (which correspond to Article 19 amendments or to claims attached to the International Preliminary Examination Report (Article 34)) during prosecution of the above-identified national phase PCT application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §1.16 or 1.14; particularly, extension of time fees.

Dated: March 28, 2006

Respectfully submitted,

By 

Mark J. Nuell, Ph.D.

Registration No.: 36,623

BIRCH, STEWART, KOLASCH & BIRCH, LLP

8110 Gatehouse Road

Suite 100 East

P.O. Box 747

Falls Church, Virginia 22040-0747

(703) 205-8000

Attorney for Applicant

Attachment(s)

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. Statement**

Novelty (N)	Claims	1-85	YES
	Claims	none	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-85	NO
Industrial applicability (IA)	Claims	1-85	YES
	Claims	none	NO

2. Citations and explanations (Rule 70.7)

D1- CA2342970 McKerracher, L. 2002/10/12
D2- EP1177796 Nakamuta et al. 2002/02/06
D3- Verschuren, et al. European Journal of Cell Biology (1997) Vol.73, pp.182-187.
D4- Imamura et al., Jpn J. Cancer Res. (2000) Vol.91, pp.811-816
D5- Yoshioka et al., FEBS Letters, Vol.372 (1995) pp.25-28.
D6- US2002/0077283 A1 Sessa, WC. June 20, 2002

D1 discloses the inactivation of Rho with the C3 toxin to facilitate the generation and sprouting of injured axons. The C3-like chimeric fusion proteins comprise a transport agent region and an active agent region, wherein said active agent region is an ADP-ribosyl transferase C3 region, in particular the ADP-ribosyl transferase C3 region from *Clostridium botulinum*. (See abstract and whole document)

D2 discloses an agent for the prophylaxis and treatment of liver diseases (hepatitis, hepatic fibrosis, hepatic cirrhosis and hepatic cancer) which contains a compound having Rho kinase activity. (See whole document)

D3 discloses that the ADP-ribosylation of Rho proteins with botulinum C3 exoenzyme inhibits invasion and shape changes of T-lymphoma cells. (See whole document)

D4 discloses the suppression of migration of rat hepatoma (MM1) cells, invasion and phagokinetic movement through the inhibition of the Rho kinase with *Clostridium botulinum* C3 exoenzyme. (See whole document)

D5 discloses that the inactivation of Rho (rho21) by ADP-ribosylase C3 results in the suppression of invasion of rat ascites hepatoma cells (MM1) through a cultured mesothelial cell monolayer (MCL). (see whole document)

D6 discloses compositions of fusion proteins comprising the antennapedia homeodomain fused to a calveolin scaffolding domain and methods of using these peptides to treat various conditions and afflictions. The peptides are useful for treating cancer in mammals, including colon, breast, melanoma, ovarian carcinoma, prostate and lung cancer. (See whole document)

Novelty:

The subject-matter of claims 1-85 is considered new under Article 33(2) PCT.

D1 differ from the methods and use of claims 1-85 in that they are directed to the treatment of cancer cells. D1 discloses methods and compositions for the inactivation of Rho kinase to facilitate the generation of injured neurons. D1 also discloses that Rho signalling antagonists are effective in the treatment of other diseases such as glaucoma, cancer cell migration and metastasis (page 12, lines 4-7). D1 also suggest that much evidence suggest efficacy of the chimeric C3 fusion proteins in the treatment of cancer cell migration (page 22, lines 26-29).

Box No. VII **Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D6 is not mentioned in the description, nor are these documents identified therein.

Supplemental Box relating to Sequence Listing

Continuation of Box No.1, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:
 - a. type of material
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material
 - ☐ on paper
 - ☒ in electronic form
 - c. time of filing/furnishing
 - ☐ contained in the international application as filed
 - ☒ filed together with the international application in electronic form
 - ☐ furnished subsequently to this Authority for the purposes of search and/or examination
 - ☐ received by this Authority as an amendment* on
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

* If item 4 in Box No. 1 applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded".

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box No. V.

Inventive step:

D1 discloses methods and compositions for the inactivation of Rho kinase to facilitate the generation of injured neurons. D1 also discloses that Rho signalling antagonists are effective in the treatment of other diseases such as glaucoma, cancer cell migration and metastasis (page 12, lines 4-7). D1 also suggest that much evidence suggest efficacy of the chimeric C3 fusion proteins in the treatment of cancer cell migration (page 22, lines 26-29).

D2, D3, D4 and D5 disclose the use of Rho kinase inhibitors for the treatment of liver diseases, inhibition of invasion and shape changes of T-lymphoma cells and the migration and invasiveness of MM1 cells. In particular D4 and D5 use *Clostridium botulinum* C3 exoenzyme to inhibit Rho kinase activity in cancer cells.

D6 discloses fusion proteins comprising a transport agent region, the antenapedia homeodomain, fused to an active agent region (the calveolin scaffolding domain) for treating cancer in mammals.

The current invention provides a method of prevention or inhibition of controlled proliferation and spreading or migration of a metastatic neoplastic cell in a mammal comprising the administration of a fusion protein comprising an active agent region (*Clostridium botulinum* C3 exotransferase, a Rho kinase inhibitor) and a transport agent region (antennapedia or TAT) and the use of said fusion protein in the treatment or prevention of cancer in a mammal.

The closest prior art document is D1 where methods and compositions for the inactivation of Rho kinase to facilitate the generation of injured neurons are disclosed.

The technical problem to be solved by the present invention is to provide a method for the prevention or inhibition of controlled proliferation and spreading or migration of a metastatic neoplastic cell in a mammal.

The solution provided by the present application is to use the fusion protein of D1 to inhibit Rho kinase activity in cancer cells. Applicant's argument received July 29, 2005 has been considered but has not been found persuasive and thus the present solution cannot be viewed as inventive.

D1 disclose the C3-exotransferase fusion protein and describes that Rho signalling antagonists are effective in the treatment of other diseases such as glaucoma, cancer cell migration and metastasis (page 12, lines 4-7). D1 also discloses that much evidence suggest efficacy of the chimeric C3 fusion proteins in the treatment of cancer cell migration (page 22, lines 26-29).

In fact, the prior art discloses the efficacy of C3 exotransferase in the inhibition of Rho kinase activity in cancer cells. D2, D3, D4 and D5 teach the use of Rho kinase inhibitors for the treatment of liver diseases, inhibition of invasion and shape changes of T-lymphoma cells and the migration and invasiveness of MM1 cells. In particular D4 and D5 use *Clostridium botulinum* C3 exoenzyme to inhibit Rho kinase activity in cancer cells. D6 teaches that fusion proteins comprising a transport agent region, the antenapedia homeodomain, fused to an active agent region (the calveolin scaffolding domain) for treating cancer in mammals. Given that the fusion protein, and the use of *Clostridium botulinum* C3 exotransferase for the inhibition of Rho kinase, is known from the prior art, that Rho kinase has been shown to be involved in cancer cell migration and invasiveness, and that fusion protein comprising a transport region, such as antennapedia or TAT, have been used to increase the bioavailability of an agent to treat cancer cells an inventive step cannot be acknowledged for the present invention; consequently claims 1-85 do not comply with Article 33(3) PCT.

Industrial Applicability

For the assessment of claims 1-29 on the question of whether or not they define subject matter that has industrial applicability, no unified criteria exists in the PCT. Further, the patentability of said claims can depend upon their formulation. Although the methods *per se* defined in claims 1-29 relate to subject matter which this Authority is not required to examine under Rule 67.1 (iv) of the PCT, the use of the compounds referred to therein for the prevention or inhibition of cell proliferation appears to represent subject matter that has industrial applicability.

Claims 30-85 appear to define subject matter that has industrial applicability under Article 33(4) of the PCT.

Box No. I Basis of the report

1. With regard to the language, this report is based on:
- ☒ the international application in the language in which it was filed
- ☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of:
- ☐ international search (Rules 12.3(a) and 23.1(b))
- ☐ publication of the international application (Rule 12.4(a))
- ☐ international preliminary examination (Rules 55.2(a) and/or 55.3(a))
2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:
- ☐ the international application as originally filed/furnished
- ☒ the description:
- ☒ pages 1-13, 15-68 and 70-88 as originally filed/furnished
- ☒ pages* 14, 69 received by this Authority on July 29, 2005
- ☐ pages* received by this Authority on _____
- ☒ the claims:
- ☐ pages as originally filed/furnished
- ☐ pages* as amended (together with any statement) under Article 19
- ☒ pages* 89-102 received by this Authority on July 29, 2005
- ☐ pages* received by this Authority on _____
- ☐ the drawings:
- ☐ pages as originally filed/furnished
- ☐ pages* received by this Authority on _____
- ☐ pages* received by this Authority on _____
- ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.
3. ☒ The amendments have resulted in the cancellation of:
- ☒ the description, pages 14 and 69 as originally filed.
- ☒ the claims, Nos. 1-85 as originally filed.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/figs
- ☐ the sequence listing (*specify*):
- ☐ any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of those sheets may be marked "superseded."

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The question whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 1-29

because:

☒ the said international application, or the said claims Nos.

relate to the following subject matter which does not require an international preliminary examination (*specify*):

Although claims 1-29 are directed to a method for the treatment of the human or animal body, which the Authority is not required to search under Rule 39.1(iv) of the PCT, the search has been carried out based on the alleged effects of the compositions referred to therein.

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos.
are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported
by the description that no meaningful opinion could be formed (*specify*):

☐ no international search report has been established for said claims Nos.

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-bis of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.

☐ See Supplemental Box for further details.

29 JULY 2005 29-07 05
IAP20 Rec'd of CTR/PTO 28 MAR 2006

aspects, wherein the therapeutically effective amount is about 1 micrograms per milliliter to about 10 micrograms per milliliter to about 50 micrograms per milliliter.

In a sixteenth aspect, this invention discloses another aspect of the previous aspects, wherein the administration is by injection, by topical application, or by implantation.

In a seventeenth aspect, this invention discloses another aspect of the previous aspects, wherein the administration is selected from the group consisting of intrarticular, intraocular, intranasal, intraneural, intradermal, intraosteal, sublingual, oral, topical, intravesical, intrathecal, intravenous, intraperitoneal, intracranial, intramuscular, subcutaneous, inhalation, atomization and inhalation, application directly into a tumor, application directly into a disease site, application directly on or into the margins remaining after resection of a tumor, enteral, enteral together with a gastroscopic procedure, and ECRP.

In an eighteenth aspect, this invention discloses another aspect of the previous aspects, wherein the polypeptidic cell-membrane transport moiety comprises a peptide containing from about 5 to about 50 amino acids.

In a nineteenth aspect, this invention discloses another aspect of the previous aspects, wherein the *Clostridium botulinum* C3 exotransferase unit comprises the amino acid sequence designated by the sequence of fusion protein BA-07.

In a twentieth aspect, this invention discloses another aspect of the previous aspects, wherein the functional analog comprises a protein exhibiting activity in the range of 50% to 500% of that of wild type *Clostridium botulinum* C3 exotransferase.

In a twenty-first aspect, this invention discloses another aspect of the previous aspects, wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier.

In a twenty-second aspect, this invention discloses another aspect of the previous aspects, wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier selected from the group consisting of poly(ethylene-co-vinyl acetate), PVA, partially hydrolyzed poly(ethylene-co-vinyl acetate), poly(ethylene-co-vinyl acetate-co-vinyl alcohol), a cross-linked poly(ethylene-co-vinyl acetate), a cross-linked

29 JULY 2005 29 - 07 .05

The % growth inhibition can be used to prepare a chart to compare the effect at different doses. The percentage growth plots are plotted, and the points where the dose response curves crossed the PG values of +50, 0, and -50 are used to calculate the GI_{50} , TGI and LC_{50} . GI_{50} , or concentration required to inhibit growth 50% is the relevant parameter for the fusion protein.

Example 7

Specific use of SRB assay to demonstrate inhibition of cell growth of human cancer cell lines

Table 3

GI_{50} (concentration for 50% inhibition of cell growth) following fusion protein treatment measured by SRB assay

Cell line	Type of Cancer	GI_{50} ($\mu\text{g/mL}$)
Caki-1	Renal	0.054
TK-10	Renal	0.52
SF-268	CNS	0.326
HOP-62	Non-SCLC	0.269
NCI-H226	Non-SCLC	48.2
HS 578T	Breast	36.6

One fusion protein of this invention, BA-07, has an effect on 4 of 6 human tumor cell lines tested with ^3H -thymidine and an effect on about 10% of the cell lines of the NCI screen. In the SRB test, it appears to have cytostatic properties; growth is inhibited compared to controls but the overall amount of protein does not decrease compared to the amount measured at time zero (T_z). These results agree with in vivo data showing that C3 transferase is not highly toxic to animals. The observed GI_{50} values are in the nanomolar to micromolar range, given a molecular weight of about 27 kDa for the fusion protein.

29 JULY 2005 29-07.05

WHAT IS CLAIMED IS:

1. A method of prevention or inhibition of uncontrolled proliferation and spreading or migration of a metastatic neoplastic cell of a cancer in a mammal, comprising administration to the mammal of a therapeutically effective amount of a pharmaceutical composition comprising a cell-permeable fusion protein conjugate comprising a polypeptidic cell-membrane transport moiety and a Clostridium botulinum C3 exotransferase unit, or a functional analog thereof.
2. A method of prevention or inhibition of uncontrolled proliferation and spreading or migration, within a resection margin of a host tissue proximal to the site of excision of a tumor of a cancer in a mammal, of a metastatic neoplastic cell residing in the resection margin, comprising administration of a therapeutically effective amount of a pharmaceutical composition comprising a cell-permeable fusion protein conjugate comprising a polypeptidic cell-membrane transport moiety and a Clostridium botulinum C3 exotransferase unit, or a functional analog thereof, said administration being directly on to the surface of the resection margin or below the surface of the resection margin or into the tissue proximal to the resection margin which remains in the mammal, said administration in a time interval prior to or subsequent to or prior to and subsequent to excision or removal of the tumor.
3. A method of prevention of growth of a tumor from a malignant cell in a host tissue in a mammal comprising administration to the mammal of a therapeutically effect amount of a pharmaceutical composition comprising a cell-permeable fusion protein conjugate comprising a polypeptidic cell-membrane transport moiety and a Clostridium botulinum C3 exotransferase unit, or a functional analog thereof, wherein the fusion protein simultaneously prevents or inhibits at least two of malignant cell migration, malignant cell proliferation, angiogenesis or tubular structure formation or capillary network growth proximal to the malignant cell, and secretion of an active metalloproteinase from the malignant cell.

29 JULY 2005 29-07.05

4. A method of prevention of growth within a resection margin of a host tissue proximal to a site of excision or removal of a first tumor of a cancer in a mammal, of a second tumor comprising a residual tumor cell of the cancer, the method comprising administration of a therapeutically effective amount of a pharmaceutical composition comprising a cell-permeable fusion protein conjugate comprising a polypeptidic cell-membrane transport moiety and a Clostridium botulinum C3 exotransferase unit, or a functional analog thereof, said administration being directly on to the surface of the resection margin or below the surface of the resection margin or into the tissue proximal to the resection margin which remains in the mammal, and said administration being in a time interval prior to, or subsequent to, or both prior to and subsequent to excision or removal of the first tumor, wherein the fusion protein simultaneously prevents or inhibits at least two of residual tumor cell migration, residual tumor cell proliferation, angiogenesis or tubular structure formation or capillary network growth proximal to the residual tumor cell, and secretion of an active metalloproteinase from the residual tumor cell.
5. The method of claim 1, wherein the fusion protein conjugate has SEQ ID NO:4.
6. The method of claim 1, wherein the cancer is selected from the group consisting of breast, brain, colon, skin, kidney, and hepatic cancer.
7. The method of claim 1, wherein the cancer is a brain tumor selected from the group consisting of glial tumors, neuron tumors, pineal gland tumors, menigeal tumors, tumors of nerve sheath, lymphomas, malformative tumors, and metastatic tumors located in the brain derived from tumors of the lung, breast, melanoma, kidney, and gastrointestinal tract.
8. The method of claim 1, wherein the cancer is a brain tumor selected from the group consisting of anaplastic astrocytoma, glioblastoma multiform, pilocytic astrocytoma, oligodendroglioma, ependymoma, myxopapillary ependymoma, subependymoma, choroid plexus papilloma, neuroblastoma, ganglioneuroblastoma, ganglioneuroma, and medulloblastoma, pineoblastoma and pineocytoma, meningioma, meningeal hemangiopericytoma, meningeal

23 JULY 2005 29-07.05

- sarcoma, Schwannoma (neurolemmoma) and neurofibroma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, primary and secondary subtypes of Hodgkin's lymphoma, primary and secondary subtypes of non-Hodgkin's lymphoma, craniopharyngioma, epidermoid cysts, dermoid cysts and colloid cysts.
9. The method of claim 1, wherein the therapeutically effective amount is about 0.001 micrograms per cc to about 50 micrograms per cc of tissue.
 10. The method of claim 1, wherein the therapeutically effective amount is about 0.0001 micrograms of fusion protein per cubic centimeter (cc) of tissue to about 100 micrograms per cubic centimeter of tissue.
 11. The method of claim 1, wherein the therapeutically effective amount is about 1 micrograms per milliliter to about 10 micrograms per milliliter to about 50 micrograms per milliliter.
 12. The method of claim 1, wherein the administration is by injection, by topical application, or by implantation.
 13. The method of claim 1, wherein the administration is selected from the group consisting of intrarticular, intraocular, intranasal, intraneural, intradermal, intraosteal, sublingual, oral, topical, intravesical, intrathecal, intravenous, intraperitoneal, intracranial, intramuscular, subcutaneous, inhalation, atomization and inhalation, application directly into a tumor, application directly into a disease site, application directly on or into the margins remaining after resection of a tumor, enteral, enteral together with a gastroscopic procedure, and ECRP.
 14. The method of claim 1, wherein the polypeptidic cell-membrane transport moiety comprises a peptide containing from about 5 to about 50 amino acids.
 15. The method of claim 1, wherein the *Clostridium botulinum* C3 exotransferase unit comprises the amino acid sequence designated by the sequence of fusion protein BA-05.

16. The method of claim 1, wherein the functional analog comprises a protein exhibiting activity in the range of 50% to 500% of that of wild type *Clostridium botulinum* C3 exotransferase.
17. The method of claim 1, wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier.
18. The method of claim 1, wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier selected from the group consisting of poly(ethylene-co-vinyl acetate), PVA, partially hydrolyzed poly(ethylene-co-vinyl acetate), poly(ethylene-co-vinyl acetate-co-vinyl alcohol), a cross-linked poly(ethylene-co-vinyl acetate), a cross-linked partially hydrolyzed poly(ethylene-co-vinyl acetate), a cross-linked poly(ethylene-co-vinyl acetate-co-vinyl alcohol), poly-D,L-lactic acid, poly-L-lactic acid, polyglycolic acid, PGA, copolymers of lactic acid and glycolic acid, polycaprolactone, polyvalerolactone, poly(anhydrides), copolymers of polycaprolactone with polyethylene glycol, copolymers of polylactic acid with polyethylene glycol, polyethylene glycol; and combinations and blends thereof.
19. The method of claim 1, wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier comprising an aqueous gelatin, an aqueous protein, a polymeric carrier, a cross-linking agent, and a combination thereof.
20. The method of claim 1, wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier comprising a matrix.
21. The method of claim 1, wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier comprising water, a pharmaceutically acceptable buffer salt, a pharmaceutically acceptable buffer solution a pharmaceutically acceptable antioxidant, ascorbic acid, one or more low molecular weight pharmaceutically acceptable polypeptide, a peptide comprising about 2 to about 10 amino acid residues, one or more pharmaceutically acceptable protein, one or more pharmaceutically acceptable

- amino acid, an essential-to-human amino acid, one or more pharmaceutically acceptable carbohydrate, one or more pharmaceutically acceptable carbohydrate-derived material, a non-reducing sugar, glucose, sucrose, sorbitol, trehalose, mannitol, maltodextrin, dextrans, cyclodextrin, a pharmaceutically acceptable chelating agent, EDTA, DTPA, a chelating agent for a divalent metal ion, a chelating agent for a trivalent metal ion, glutathione, pharmaceutically acceptable nonspecific serum albumin, and combinations thereof.
22. The method of claim 1, wherein the pharmaceutical composition is sterile.
23. The method of claim 1, wherein the pharmaceutical composition is sterilizable.
24. The method of claim 1, wherein the pharmaceutical composition is sterilized.
25. The method of claim 1, wherein the pharmaceutical composition is in a vial in a unit dosage amount or in an integral multiple of a unit dosage amount.
26. The method of claim 1, wherein the pharmaceutical composition is dried.
27. The method of claim 1, wherein the pharmaceutical composition comprises a dehydrated matrix.
28. The method of claim 1, wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier.
29. The method of claim 1, wherein the pharmaceutical composition comprises a fusion protein in a lyophilized matrix.
30. Use of a pharmaceutical composition comprising a cell-permeable fusion protein conjugate comprising a polypeptidic cell-membrane transport moiety and a *Clostridium botulinum* C3 exotransferase unit, or a functional analog thereof, for preventing or inhibiting uncontrolled proliferation and spreading or migration of a metastatic neoplastic cell of a cancer in a mammal.

29 JULY 2005 29-07.05

31. Use of a pharmaceutical composition comprising a cell-permeable fusion protein conjugate comprising a polypeptidic cell-membrane transport moiety and a Clostridium botulinum C3 exotransferase unit, or a functional analog thereof, for preventing or inhibiting uncontrolled proliferation and spreading or migration, within a resection margin of a host tissue proximal to the site of excision of a tumor of a cancer in a mammal, of a metastatic neoplastic cell residing in the resection margin.
32. Use of a pharmaceutical composition comprising a cell-permeable fusion protein conjugate comprising a polypeptidic cell-membrane transport moiety and a Clostridium botulinum C3 exotransferase unit, or a functional analog thereof, for preventing growth of a tumor from a malignant cell in a host tissue in a mammal, wherein the fusion protein simultaneously prevents or inhibits at least two of malignant cell migration, malignant cell proliferation, angiogenesis or tubular structure formation or capillary network growth proximal to the malignant cell, and secretion of an active metalloproteinase from the malignant cell.
33. Use of a pharmaceutical composition comprising a cell-permeable fusion protein conjugate comprising a polypeptidic cell-membrane transport moiety and a Clostridium botulinum C3 exotransferase unit, or a functional analog thereof, for preventing growth within a resection margin of a host tissue proximal to a site of excision or removal of a first tumor of a cancer in a mammal, of a second tumor comprising a residual tumor cell of the cancer, wherein the fusion protein simultaneously prevents or inhibits at least two of residual tumor cell migration, residual tumor cell proliferation, angiogenesis or tubular structure formation or capillary network growth proximal to the residual tumor cell, and secretion of an active metalloproteinase from the residual tumor cell.
34. The use of any one of claims 30 to 33, wherein the fusion protein conjugate has SEQ ID NO:4.
35. The use of claim 30, 31 or 33, wherein the cancer is selected from the group

29 JULY 2005 29-07.05

consisting of breast, brain, colon, skin, kidney, and hepatic cancer.

36. The use of claim 35, wherein the cancer is a brain tumor selected from the group consisting of glial tumors, neuron tumors, pineal gland tumors, meningeal tumors, tumors of nerve sheath, lymphomas, malformative tumors, and metastatic tumors located in the brain derived from tumors of the lung, breast, melanoma, kidney, and gastrointestinal tract.

37. The use of claim 35, wherein the cancer is a brain tumor selected from the group consisting of anaplastic astrocytoma, glioblastoma multiform, pilocytic astrocytoma, oligodendroglioma, ependymoma, myxopapillary ependymoma, subependymoma, choroid plexus papilloma, neuroblastoma, ganglioneuroblastoma, ganglioneuroma, and medulloblastoma, pineoblastoma and pineocytoma, meningioma, meningeal hemangiopericytoma, meningeal sarcoma, Schwannoma (neurolimmoma) and neurofibroma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, primary and secondary subtypes of Hodgkin's lymphoma, primary and secondary subtypes of non-Hodgkin's lymphoma, craniopharyngioma, epidermoid cysts, dermoid cysts and colloid cysts.

38. The use of any one of claims 30 to 37, wherein the pharmaceutical composition is formulated for a dosage form of about 0.001 micrograms per cc to about 50 micrograms per cc of tissue.

39. The use of any one of claims 30 to 37, wherein the pharmaceutical composition is formulated for a dosage form of about 0.0001 micrograms of fusion protein per cubic centimeter (cc) of tissue to about 100 micrograms per cubic centimeter of tissue.

40. The use of any one of claims 30 to 37, wherein the pharmaceutical composition is formulated for a dosage form of about 1 micrograms per milliliter to about 10 micrograms per milliliter to about 50 micrograms per milliliter.

41. The use of any one of claims 30 to 40, wherein the pharmaceutical

29 JULY 2005 29-07.05

composition is formulated for injection, topical application, or implantation.

42. The use of claim 1, any one of claims 30 to 40, wherein the pharmaceutical composition is formulated for an administration mode selected from the group consisting of intrarticular, intraocular, intranasal, intraneural, intradermal, intraosteal, sublingual, oral, topical, intravesical, intrathecal, intravenous, intraperitoneal, intracranial, intramuscular, subcutaneous, inhalation, atomization and inhalation, application directly into a tumor, application directly into a disease site, application directly on or into the margins remaining after resection of a tumor, enteral, enteral together with a gastroscopic procedure, and ECRP.
43. The use of any one of claims 30 to 42, wherein the polypeptidic cell-membrane transport moiety comprises a peptide containing from about 5 to about 50 amino acids.
44. The use of any one of claims 30 to 43, wherein the Clostridium botulinum C3 exotransferase unit comprises the amino acid sequence SEQ ID NO:4 of fusion protein BA-05.
45. The use of any one of claims 30 to 44, wherein the functional analog comprises a protein exhibiting activity in the range of 50% to 500% of that of wild type Clostridium botulinum C3 exotransferase.
46. The use of any one of claims 30 to 45, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.
47. The use of claim 46, wherein the pharmaceutically acceptable carrier is selected from the group consisting of poly(ethylene-co-vinyl acetate), PVA, partially hydrolyzed poly(ethylene-co-vinyl acetate), poly(ethylene-co-vinyl acetate-co-vinyl alcohol), a cross-linked poly(ethylene-co-vinyl acetate), a cross-linked partially hydrolyzed poly(ethylene-co-vinyl acetate), a cross-linked poly(ethylene-co-vinyl acetate-co-vinyl alcohol), poly-D,L-lactic acid, poly-L-lactic acid, polyglycolic acid, PGA, copolymers of lactic acid and glycolic acid, polycaprolactone, polyvalerolactone, poly (anhydrides),

29 JULY 2005 29 -07 .05

copolymers of polycaprolactone with polyethylene glycol, copolymers of polylactic acid with polyethylene glycol, polyethylene glycol; and combinations and blends thereof.

48. The use of claim 46, wherein the pharmaceutically acceptable carrier comprises an aqueous gelatin, an aqueous protein, a polymeric carrier, a cross-linking agent, and a combination thereof.

49. The use of claim 46, wherein the pharmaceutically acceptable carrier comprises a matrix.

50. The use of claim 46, wherein the pharmaceutically acceptable carrier comprises at least one carrier selected from the group consisting of water, a pharmaceutically acceptable buffer salt, a pharmaceutically acceptable buffer solution, a pharmaceutically acceptable antioxidant, ascorbic acid, a low molecular weight pharmaceutically acceptable polypeptide, a peptide comprising about 2 to about 10 amino acid residues, a pharmaceutically acceptable protein, a pharmaceutically acceptable amino acid, an essential-to-human amino acid, a pharmaceutically acceptable carbohydrate, a pharmaceutically acceptable carbohydrate-derived material, a non-reducing sugar, glucose, sucrose, sorbitol, trehalose, mannitol, maltodextrin, dextrans, cyclodextrin, a pharmaceutically acceptable chelating agent, EDTA, DTPA, a chelating agent for a divalent metal ion, a chelating agent for a trivalent metal ion, glutathione, and pharmaceutically acceptable nonspecific serum albumin.

51. The use of any one of claims 30 to 50, wherein the pharmaceutical composition is sterile.

52. The use of any one of claims 30 to 50, wherein the pharmaceutical composition is sterilizable.

53. The use of any one of claims 30 to 50, wherein the pharmaceutical composition is sterilized.

54. The use of any one of claims 30 to 53, wherein the pharmaceutical

29 JULY 2005 29-07.05

composition is in a vial in a unit dosage amount or in an integral multiple of a unit dosage amount.

55. The use of any one of claims 30 to 54, wherein the pharmaceutical composition is dried.
56. The use of any one of claims 30 to 54, wherein the pharmaceutical composition comprises a dehydrated matrix.
57. The use of any one of claims 30 to 54, wherein the pharmaceutical composition comprises a fusion protein in a lyophilized matrix.
58. Use of a pharmaceutical composition comprising a cell-permeable fusion protein conjugate comprising a polypeptidic cell-membrane transport moiety and a Clostridium botulinum C3 exotransferase unit, or a functional analog thereof, for the manufacture of a medicament for preventing or inhibiting uncontrolled proliferation and spreading or migration of a metastatic neoplastic cell of a cancer in a mammal.
59. Use of a pharmaceutical composition comprising a cell-permeable fusion protein conjugate comprising a polypeptidic cell-membrane transport moiety and a Clostridium botulinum C3 exotransferase unit, or a functional analog thereof, for the manufacture of a medicament for preventing or inhibiting uncontrolled proliferation and spreading or migration, within a resection margin of a host tissue proximal to the site of excision of a tumor of a cancer in a mammal, of a metastatic neoplastic cell residing in the resection margin.
60. Use of a pharmaceutical composition comprising a cell-permeable fusion protein conjugate comprising a polypeptidic cell-membrane transport moiety and a Clostridium botulinum C3 exotransferase unit, or a functional analog thereof, for the manufacture of a medicament for preventing growth of a tumor from a malignant cell in a host tissue in a mammal, wherein the fusion protein simultaneously prevents or inhibits at least two of malignant cell migration, malignant cell proliferation, angiogenesis or tubular structure formation or capillary network growth proximal to the malignant cell, and secretion of an

29 JULY 2005 29 -07 .05

active metalloproteinase from the malignant cell.

61. Use of a pharmaceutical composition comprising a cell-permeable fusion protein conjugate comprising a polypeptidic cell-membrane transport moiety and a Clostridium botulinum C3 exotransferase unit, or a functional analog thereof, for the manufacture of a medicament for preventing growth within a resection margin of a host tissue proximal to a site of excision or removal of a first tumor of a cancer in a mammal, of a second tumor comprising a residual tumor cell of the cancer, wherein the fusion protein simultaneously prevents or inhibits at least two of residual tumor cell migration, residual tumor cell proliferation, angiogenesis or tubular structure formation or capillary network growth proximal to the residual tumor cell, and secretion of an active metalloproteinase from the residual tumor cell.
62. The use of any one of claims 58 to 61, wherein the fusion protein conjugate has SEQ ID NO:4.
63. The use of claim 58, 59 or 61, wherein the cancer is selected from the group consisting of breast, brain, colon, skin, kidney, and hepatic cancer.
64. The use of claim 63, wherein the cancer is a brain tumor selected from the group consisting of glial tumors, neuron tumors, pineal gland tumors, meningeal tumors, tumors of nerve sheath, lymphomas, malformative tumors, and metastatic tumors located in the brain derived from tumors of the lung, breast, melanoma, kidney, and gastrointestinal tract.
65. The use of claim 63, wherein the cancer is a brain tumor selected from the group consisting of anaplastic astrocytoma, glioblastoma multiform, pilocytic astrocytoma, oligodendroglioma, ependymoma, myxopapillary ependymoma, subependymoma, choroid plexus papilloma, neuroblastoma, ganglioneuroblastoma, ganglioneuroma, and medulloblastoma, pineoblastoma and pineocytoma, meningioma, meningeal hemangiopericytoma, meningeal sarcoma, Schwannoma (neurolemmoma) and neurofibroma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, primary and secondary subtypes of

29 JULY 2005 29-07.05

Hodgkin's lymphoma, primary and secondary subtypes of non-Hodgkin's lymphoma, craniopharyngioma, epidermoid cysts, dermoid cysts and colloid cysts.

66. The use of any one of claims 58 to 65, wherein the pharmaceutical composition is formulated for a dosage form of about 0.001 micrograms per cc to about 50 micrograms per cc of tissue.
67. The use of any one of claims 58 to 65, wherein the pharmaceutical composition is formulated for a dosage form of about 0.0001 micrograms of fusion protein per cubic centimeter (cc) of tissue to about 100 micrograms per cubic centimeter of tissue.
68. The use of any one of claims 58 to 65, wherein the pharmaceutical composition is formulated for a dosage form of about 1 micrograms per milliliter to about 10 micrograms per milliliter to about 50 micrograms per milliliter.
69. The use of any one of claims 58 to 68, wherein the pharmaceutical composition is formulated for injection, topical application, or implantation.
70. The use of claim 1, any one of claims 58 to 68, wherein the pharmaceutical composition is formulated for an administration mode selected from the group consisting of intrarticular, intraocular, intranasal, intraneural, intradermal, intraosteal, sublingual, oral, topical, intravesical, intrathecal, intravenous, intraperitoneal, intracranial, intramuscular, subcutaneous, inhalation, atomization and inhalation, application directly into a tumor, application directly into a disease site, application directly on or into the margins remaining after resection of a tumor, enteral, enteral together with a gastroscopic procedure, and ECRP.
71. The use of any one of claims 58 to 70, wherein the polypeptidic cell-membrane transport moiety comprises a peptide containing from about 5 to about 50 amino acids.

29 JULY 2005 29-07.05

72. The use of any one of claims 58 to 71, wherein the Clostridium botulinum C3 exotransferase unit comprises the amino acid sequence SEQ ID NO:4 of fusion protein BA-05.
73. The use of any one of claims 58 to 72, wherein the functional analog comprises a protein exhibiting activity in the range of 50% to 500% of that of wild type Clostridium botulinum C3 exotransferase.
74. The use of any one of claims 58 to 73, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier:
75. The use of claim 74, wherein the pharmaceutically acceptable carrier is selected from the group consisting of poly(ethylene-co-vinyl acetate), PVA, partially hydrolyzed poly(ethylene-co-vinyl acetate), poly(ethylene-co-vinyl acetate-co-vinyl alcohol); a cross-linked poly(ethylene-co-vinyl acetate), a cross-linked partially hydrolyzed poly(ethylene-co-vinyl acetate), a cross-linked poly(ethylene-co-vinyl acetate-co-vinyl alcohol), poly-D,L-lactic acid, poly-L-lactic acid, polyglycolic acid, PGA, copolymers of lactic acid and glycolic acid, polycaprolactone, polyvalerolactone, poly (anhydrides), copolymers of polycaprolactone with polyethylene glycol, copolymers of polylactic acid with polyethylene glycol, polyethylene glycol; and combinations and blends thereof.
76. The use of claim 74, wherein the pharmaceutically acceptable carrier comprises an aqueous gelatin, an aqueous protein, a polymeric carrier, a cross-linking agent, and a combination thereof.
77. The use of claim 74, wherein the pharmaceutically acceptable carrier comprises a matrix.
78. The use of claim 74, wherein the pharmaceutically acceptable carrier comprises at least one carrier selected from the group consisting of water, a pharmaceutically acceptable buffer salt, a pharmaceutically acceptable buffer solution, a pharmaceutically acceptable antioxidant, ascorbic acid, a low molecular weight pharmaceutically acceptable polypeptide, a peptide

comprising about 2 to about 10 amino acid residues, a pharmaceutically acceptable protein, a pharmaceutically acceptable amino acid, an essential-to-human amino acid, a pharmaceutically acceptable carbohydrate, a pharmaceutically acceptable carbohydrate-derived material, a non-reducing sugar, glucose, sucrose, sorbitol, trehalose, mannitol, maltodextrin, dextrans, cyclodextrin, a pharmaceutically acceptable chelating agent, EDTA, DTPA, a chelating agent for a divalent metal ion, a chelating agent for a trivalent metal ion, glutathione, and pharmaceutically acceptable nonspecific serum albumin.

79. The use of any one of claims 58 to 78, wherein the pharmaceutical composition is sterile.
80. The use of any one of claims 58 to 78, wherein the pharmaceutical composition is sterilizable.
81. The use of any one of claims 58 to 78, wherein the pharmaceutical composition is sterilized.
82. The use of any one of claims 58 to 81, wherein the pharmaceutical composition is in a vial in a unit dosage amount or in an integral multiple of a unit dosage amount.
83. The use of any one of claims 58 to 82, wherein the pharmaceutical composition is dried.
84. The use of any one of claims 58 to 82, wherein the pharmaceutical composition comprises a dehydrated matrix.
85. The use of any one of claims 58 to 82, wherein the pharmaceutical composition comprises a fusion protein in a lyophilized matrix.